Lab-Scale Flash Purification of Synthetic Lipid Facilitated by Evaporative Light Scattering Detection

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Introduction

Synthetic lipids and lipid nanoparticles (LNPs) have been used for mRNA-based drug administration for several years. The lipid mixtures typically contain four compounds, including cholesterol, a phospholipid, a PEGylated lipid, and a synthetic cationic lipid. This application note addresses the purification of a specific synthetic polar lipid, ALC-0315[1], Figure 1.

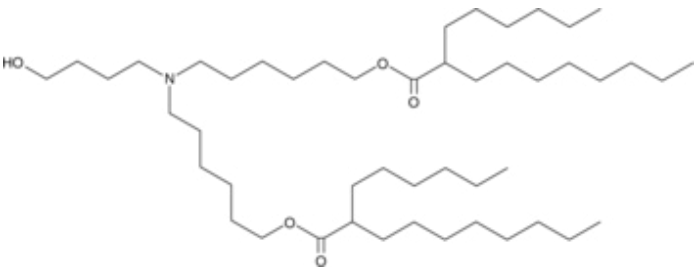

Figure 1. ALC-0315 structure.

The synthesis of ALC-0315 is a multi-step process, also yielding several byproducts or impurities that require removal by normal phase flash chromatography. However, due to the poor UV absorption by these lipids, their detection during chromatography is challenging. To address this issue and detect the synthetic lipids effectively, an evaporative light scattering detector (ELSD) was added into a Biotage® Selekt Enkel flash chromatography system.

Experimental and Results

After various purification methods were tested, a heptane/ethyl acetate gradient and a Biotage® Sfär KP-Amino column was found to provide a significant separation between ALC-0315 and its synthetic byproducts.

Chromatographic Conditions

For purification, Biotage® Selekt Enkel flash purification system together with Biotage® Selekt ELSD module was used with a 5-gram Biotage® Sfär KP-Amino column.

Column:	Biotage® Sfär KP-Amino, 5-gram
Solvent A:	Heptane
Solvent B:	Ethyl acetate
Gradient:	5% B 1 CV 5 - 40% B 10 CV 40% B for 2 CV
Flow rate:	15 mL/min
UV Detection:	UV λ-all 200-400 nm
ELSD solvent:	Acetone
ELSD temp:	36 °C
ELSD N2:	1.5 bar

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Chromatographic Results

The linear gradient generated an excellent separation (ΔCV =2) of the cationic lipid ALC-0315 from the less polar byproducts, Figure 2.

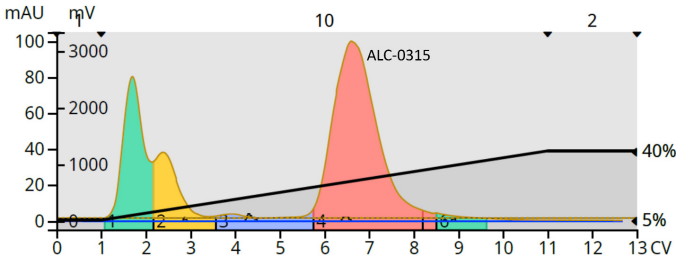

Figure 2. Crude ALC-0315 reaction mixture purification using 5 - 40% ethyl acetate in heptane linear gradient.

ELSD was necessary for lipid detection, because ethyl acetate absorbs UV light within the same wavelength range as the lipids (200-215 nm), effectively masking any lipid UV response.

Further Optimization with a Step Gradient

While this linear gradient provided a good purification, it consumed 13 column volumes (CV) of solvent (117 mL), so further enhancement was explored to reduce solvent consumption.

Column:	Biotage® Sfär KP-Amino, 5-gram
Solvent A:	Heptane
Solvent B:	Ethyl acetate
Gradient:	10% B 3 CV 20% B 8 CV
Flow rate:	15 mL/min
UV Detection:	UV λ-all 200-400 nm
ELSD solvent:	Acetone
ELSD temp:	36 °C
ELSD N2:	1.5 bar

The step gradient provided an equally successful purification and also reduced solvent consumption by 15%, 2 CV of solvent (18 mL), Figure 3.

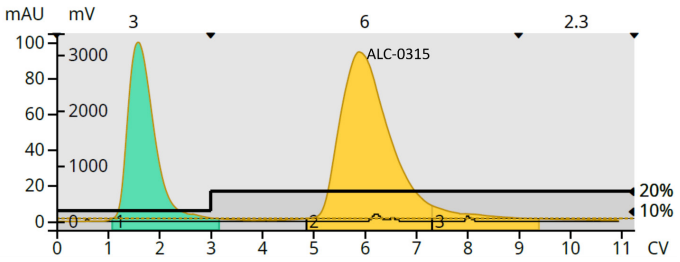

Figure 3. Crude lipid reaction mixture purification using a step gradient.

Mass analysis of the ALC-0315 peak verified its purity, Figure 4.

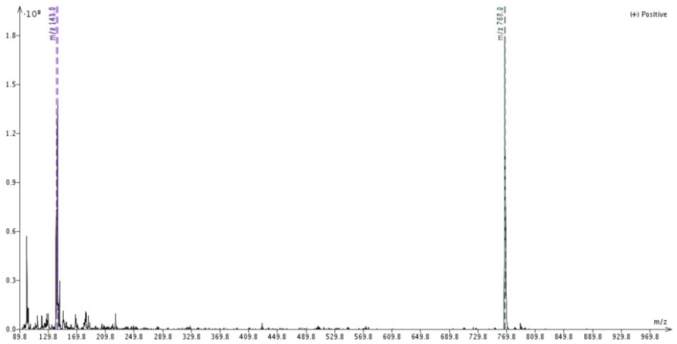

Figure 4. Purified ALC-0315 mass analysis. The m/z of 141 is from the mass detector make-up solvent.

Conclusions

Biotage® Selekt ELSD used with Biotage® Selekt Enkel flash purification system easily detected the separated lipids from a crude reaction mixture, while UV detection provided no significant value.

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